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A Biosystematical Investigation on *Muscari* species in Iran

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Abstract: The present study tends to investigate the anatomy and palynology of *Muscari* species in Iran. To conduct the comparative study of anatomy characters, sections from root, stem and leaf were prepared using microtom and differential staining. Among the organs, mesophyll structure of the leaf displayed a variety among the subgenera. For the palynology study, too, a comparative investigation on the species showed some differences in size of sexine areas and grooves between them were among the subgenera.

Key words: Anatomy, *Botryanthus*, Iran, *Leopoldia*, *Muscari*, palynology, *Pseudomuscari*, subgenera

INTRODUCTION

Muscari Mill. (Miller, 1754), from Liliaceae, Lilioideae subfamily and Scilleae tribe (Engler, 1887) has a pan-Mediterranean origin having numerous variety of species, it is expanded from Mediterranean to Europe, North Africa and to West Asia (Boissier, 1854; Post, 1935; Parsa, 1950; Davis and Stuart, 1966; Garbari and Greuter, 1970; Davis and Stuart, 1980; Pignatti, 1982; Speta, 1982; Davis, 1984; Townsend and Guest, 1985; Assadi, 1986; Rechinger, 1990). *Muscari* has 50 species around the world, about ten of which are found in Iran. Iranian species belong to three subgenera: *Leopoldia*, *Botryanthus*, *Pseudomuscari*.

The purpose of present biosystematical study is to investigate the relationship between the inner and outer structural changes. This respect we collected fresh plants from their localities and reviewed some dry samples from TARI and IRAN herbariums. In terms of comparative anatomic studies on *Muscari*, there hasn't been anything reported, except for the presence of contractile root in *M. parviflorum* (Fahn, 1990). Then we prepared some cross sections of different organs like root, stem and leaf. We also did stem maceration. There were found some differences in the form of leaf mesophyll tissue among the subgenera. In the palynological study, the comparison were made among the pollen grains of *Muscari*. In this part of study, pollen grains were extracted and acetolised, to study the size, form (shape) and ornamentation of the pollen through SEM and LM. Significant differences among the subgenera were observed concerning depth, width of muri and shape of luminas.

MATERIALS AND METHODS

As for the anatomic study, fresh samples were collected from the north, east, west and center of Iran to be studied along with dry samples from TARI and IRAN herbariums February until June 2003, 2004 (Table 1). Having been fixed in FAA, then they were dehydrated with ethanol and later, some slices were prepared with microtom. The section-12 micron thick- were then stained with Safranin and Fast-green (Johnson, 1940; Chamberlain, 1990). For the stem maceration, this organ was placed in Jeffery solution for 6 h to soften. In the palynological study, the pollen were extracted from the anther and dehydrated by Glacial acetic acid, then, acetolised and finally studied by SEM and LM (Erdtman, 1952; Moore *et al.*, 1991). Pollen terminology was adapted from Punt (Punt *et al.*, 1994).

RESULTS

Anatomic results: The results from the anatomic studies demonstrated the root with the following tissues:

- Some parenchymatous layers with small cells under the epidermis
- Some parenchymatous layers with large cells and idioblasts containing raphid and helical thickening vessel (Fig. 1)

In the stem, we found the following tissues:

- Some (several) parenchymatous layers with small cells under the epidermis

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Table 1: Species, their locality and voucher specimen

Species	Locality	Voucher No.
Muscari comosum	Azarbaijan	60263
M. caucasicum	Khorassan	16865
M. tenuiflorum	Lorestan	965
M. longipes	Hamedan	1678
M. commutatum	Lorestan	2988
M. neglectum	Kurdestan	19999
M. armeniacum var. *szovitzianum*	Azarbaijan	24968
M. microstomum	Zanjan	30106
M. pseudomuscari	Mazandaran	19162
M. inconstrictum	Gilan	60109

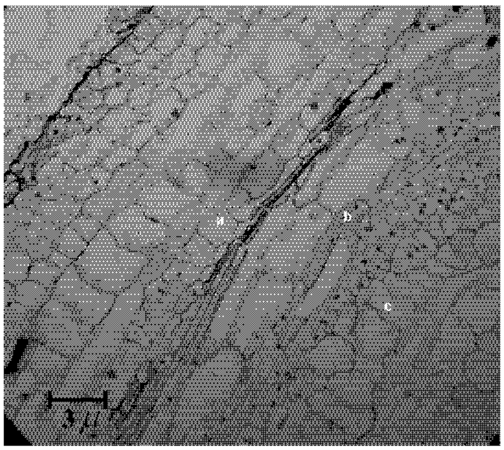

Fig. 1: Longitudinal section of root, (a) parenchyma with small cell, (b) helical thickening vessel and (c) parenchyma with large cell (x 940)

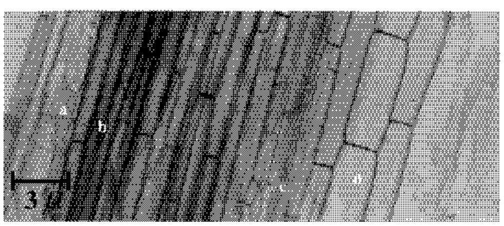

Fig. 2: Longitudinal section of stem, (a) parenchyma with small cell, (b) sclerenchyma, (c) helical thickening tracheid and (d) parenchyma with large cell (x940)

- Some sclerenchymatous layers with small cells
- Some parenchymatous layers with large cells, idioblast and helical thickening tracheids (Fig. 2)

The results from stem maceration confirmed the presence of bipolar sclereids which had created some branches in the intercellular space.

The results from the anatomic study of the leaf showed two kind of mesophyll: the upper, the medial and the lower, which the upper and the lower ones are identical in that both are made of two cell layers. However, the form of leaf mesophyll cells are different among the subgenera. The form of both upper and lower mesophylls tissue in *M. comosum* (L.) Mill (Fig. 3), *M. caucasicum* (Griseb.) Baker, *M. longipes* Boiss from subgen. *Leopoldia* are palisade, with little intercellular space.

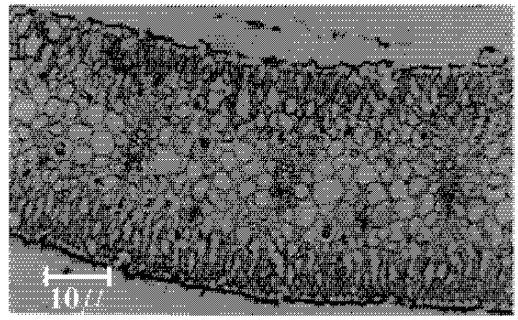

Fig. 3: Cross section of leaf of *M. comosum* from subgen *Leopoldia* with palisade mesophyll (x416)

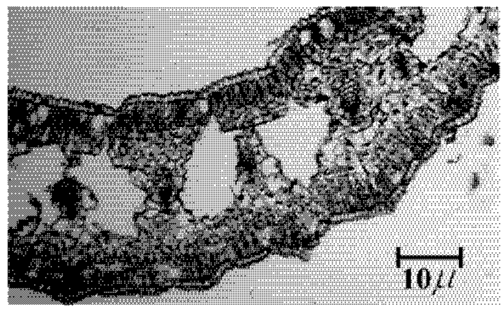

Fig. 4: Cross section of leaf of *M. tenuiflorum* with large intercellular in medial mesophyll (x416)

In *M. tenuiflorum* from subgen. *Leopoldia*, both mesophylls tissue are palisade, but there is no intercellular space. Medial mesophyll tissue is wider and has some small cells with huge intercellular spaces (Fig. 4).

In *M. neglectum* Guss., *M. armeniacum* Baker var. *szovitzianum* Stuart. from subgen. *Botryanthus*, the shapes of upper and lower mesophyll tissues range from elliptical to spherical and are totally narrower than the medial mesophyll. Also in *M. neglectum,* medial mesophyll has huge intercellular spaces (Fig. 5). Both the upper and lower mesophyll tissue in *M. commutatum* Guss., *M. microstomum* Davis and Stuart (Fig. 6) from subgen. *Bothyanthus,* are spherical to elliptical. Medial mesophyll in *M. microstomum,* haven't intercellular spaces. In *M. pseudomuscari* from subgen. *Pseudomuscari*, both upper and lower mesophyll tissues show elliptical to spherical and the thickness of the medial mesophyll is the same as the two others (Fig. 7).

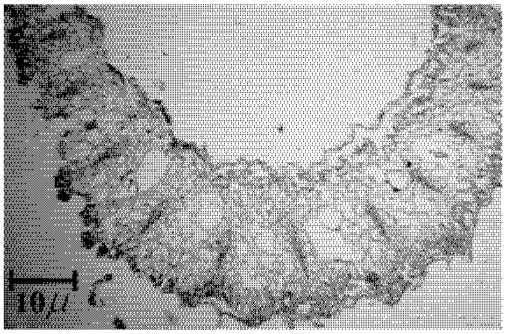

Fig. 5: Cross section of leaf of *M. neglectum* from subgen. *Botryanthus* with elliptical-spherical mesophyll. Arrow shows intercellular space (x416)

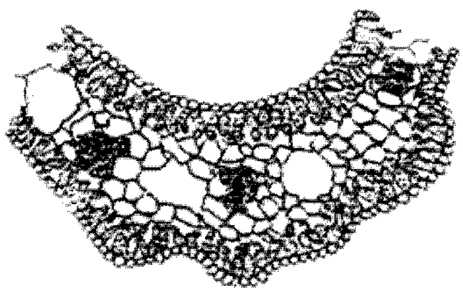

Fig. 6: Cross section of leaf of *M. microstomum* with elliptical mesophyll (x416)

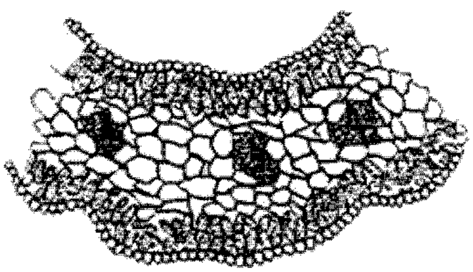

Fig. 7: Cross section of leaf of *M. pseudomuscari* from subgen. *Pseudomuscari* with spherical-elliptical mesophyll (x416)

Palynology results: The pollen grains are elliptical, monosulcate, bilateral symmetric, distal-polar, semi-tectate, without annulus, negative reticulate. The colpus is very long, curving around the ends of the grain so that it is almost divided into two lobes like the halves of a bivalve mollusc shell (Woudenhouse, 1935; Zavada, 1983; Fumess and Rudall, 1999; Harley and Zavada, 2000). Negative sexine means a pattern of ornamentation in which sexine areas are separated by narrow, reticulately

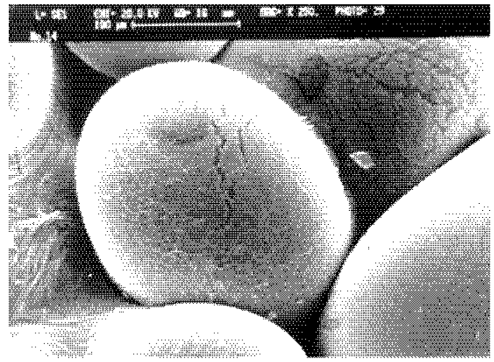

Fig. 8: Scanning electromicrograph of pollen. of *M. comosum* with small sexine areas, narrow and shallow grooves (SEM x250)

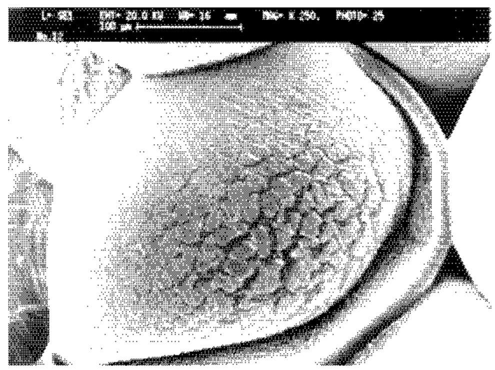

Fig. 9: Scanning electromicrograph of pollen of *M. tenuiflorum* with large sexine areas, deep and wide grooves (SEM x250)

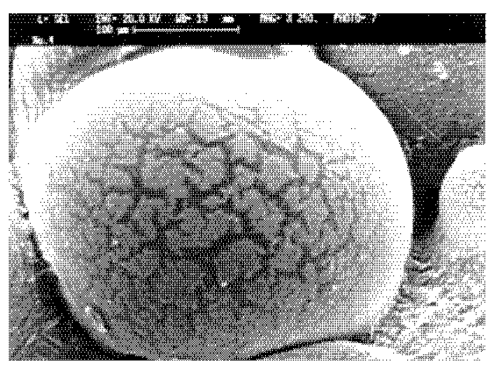

Fig. 10: Scanning electromicrograph of pollen of *M. commutatum* with large sexine areas, deep and wide grooves (SEM x250)

arranged grooves. The pollen of *M. comosum* (Fig. 8) and *M. caucasicum* from subgen. *Leopoldia* has small sexine area, smooth surface with narrow and shallow grooves.

Table 2: The ratio of P/E (polar axis length/equatorial axis length)

Species	E	P	M_{max}	M_{min}	P_{max}	P_{min}	P/E
M. caucasicum	68.25	99.75	78.75	63.00	126.00	78.75	1.46
M. comosum	75.60	116.55	78.75	63.00	126.00	94.50	1.54
M. tenuiflorum	74.00	107.10	78.75	63.00	126.00	94.50	1.44
M. longipes	94.50	116.55	110.25	78.75	141.75	110.25	1.23
M. commutatum	54.65	78.75	63.00	31.50	94.50	63.00	1.44
M. neglectum	47.25	63.00	47.25	47.25	78.75	63.00	1.33
M. microstomum	47.25	59.85	63.00	31.50	63.00	31.50	1.26
M. armeniacum var. *szovitzianum*	69.30	97.65	94.50	63.00	110.25	94.00	1.40
M. inconstrictum	40.95	63.00	47.25	31.50	78.75	47.25	1.53
M. pseudomuscari	69.30	103.90	110.25	94.50	94.50	63.00	1.58

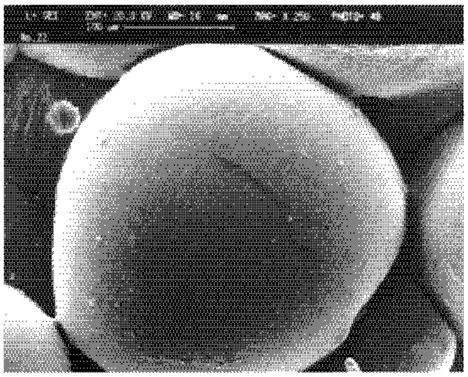

Fig. 11: Scanning of electromicrograph of pollen of *M. neglectum* with soft surface, small sexine areas, narrow and shallow grooves (SEM x250)

Fig. 12: Scanning electromicrograph of pollen. *M. pseudomuscari* with large sexine areas, deep and wide grooves (SEM x250)

The pollen of *M. tenuiflorum* (Fig. 9) and *M. longipes* from the same subgenus (*Leopoldia*) have large sexine area with deep and wide grooves. In *M. commutatum* (Fig. 10), *M. microstomum* and *M. inconstrictum* from subgen. *Botryanthus*, the pollen have large sexine area with wide and deep grooves, too. In contrast to *M. neglectum* (Fig. 11), *M. armeniacum* var. *szovitziamum* which go to the same subgenus, have a pollen with smooth surface, small sexine areas with very narrow and shallow grooves. In *M. pseudomuscari* from subgen. *Pseudomuscari*, the pollen has relatively large sexine are with wide and deep grooves (Fig. 12). Furthermore, P/E ratio (polaraxis length/equatorial axis length) were calculated. The maximum ratio was related to *M. pseudomuscari* with 1.58 while the minimum was 1.23 for *M. longipes* (Table 2).

DISCUSSION

As the results from the anatomic studies about *Muscari* suggest, we can state the anatomic structure of the roots and stems in all subgenera under the investigation, were similar while significant differences in the shape of the leaf mesophyll tissues were observed. For example, the upper and lower mesophyll tissues in subgen. *Leopoldia* were palisade, while in subgen. *Botryanthus*, they were elliptical to spherical and in subgen. *Pseudomuscari*, spherical to elliptical. The thickness of medial mesophyll was either the same as the other or wider than them in all subgenera. Sometimes some intercellular spaces could be observed in medial mesophyll tissues. As for the taxonomic position, the subgenera fall in the following order: *Leopoldia*, *Botryanthus*, *Pseudomuscari* (Stuart, 1965). Regarding the shape of the leaf mesophyll tissues, some changes were also observed: e.g., in subgen. *Leopoldia* there was palisade which changed to elliptical in subgen. *Botryanthus* and to spherical in subgen. *Pseudomuscari*. Thus, there must be a relationship between the taxonomic position of subgenera and the changes of leaf mesophyll tissues form.

Studying of the pollen of subgen. *Leopoldia*, two types of pollen ornamentations were observed:

- Pollen with small sexine areas, smooth surface, narrow and shallow grooves
- Pollen which had large sexine areas, wide and deep grooves

In subgen. *Botryanthus*-as the above subgenus-we also had both types, but in subgen. *Pseudomuscari*, the pollen had large sexine areas with wide and deep grooves.

In subgen. *Leopoldia*, two types of pollen ornamentation were observed. Besides, in the species which are morphologically identical in pairs, the ornamentation were also the same. In subgen. *Botryanthus*, too, we had two types of ornamentation. One of them was specific to *M. neglectum* and the other one which is similar to that of subgen. *Pseudomuscari*. Other three species e.g., *M. microstomum*, *M. inconstrictum* and *M. commutatum* had the same pollen ornamentation. This could lie in the fact that the three species are morphologically similar to *M. pseudomuscari*. In that they have almost no clear contraction at the corolla throat.

In conclusion, we can say that the similarity of their ornamentation, to some extant, related to their morphological similarity. However, one can not attribute such similarity to recognize taxonomic situation of species.

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